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TITLE: Defining B. Anthracis Protective Antigen Antigenic Domains

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14. ABSTRACT

The US Army faces the threat of biological warfare with Bacillus anthracis spores. Although there is a vaccine available (AVA vaccine) this formulation is poorly immunogenic, has questionable efficacy in some hosts, and is associated with significant side effects. The most important antigen in this vaccine is a component of anthrax toxin known as protective antigen (PA). However, this protein can also elicit potentially deleterious antibodies. This research program seeks dissect the PA molecule to identify protein segments that are both immunogenic and elicit useful antibodies. Hence our goal is to define regions in the PA molecule that are both immunogenic and elicit only 'good' antibodies. To do so we have developed a peptide ELISA incorporating overlapping peptides representing the entire molecule and used this assay to identify peptides reactive with protective monoclonal antibodies. Hence, we have defined the first linear peptide protective epitopes in PA. One of these peptides has been assembled into a vaccine formulation and was found to be immunogenic. We are hopeful that the identification of peptides that can elicit protective antibodies could lead to a new approach for designing a more effective vaccine for protection against anthrax.

15. SUBJECT TERMS
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Annual Progress Report

Introduction

Bacillus anthracis is a gram positive soil bacterium that is a powerful agent for bioterrorism and biological warfare. There is an acute need for a safer and more effective vaccine against *Bacillus anthracis* than the currently available US licensed Anthrax vaccine absorbed (AVA) vaccine. The AVA vaccine is poorly immunogenic, has questionable efficacy in some hosts, and is associated with significant side effects. The most important antigen in this vaccine is a component of anthrax toxin known as protective antigen (PA). PA can elicit toxin-neutralizing antibodies that are protective against experimental toxin challenge and *B. anthracis* infection. A recent attempt to generate a second-generation vaccine based on the use of recombinant PA (rPA) failed because of protein stability issues. This application proposes to dissect the PA molecule to identify protein segments that are both immunogenic and elicit useful antibodies. Creating an antigenic map of rPA is an important endeavor because this protein can elicit protective, non-protective, and even some antibodies with the potential for enhancing disease. Current immunological knowledge is not sufficient to predict useful B cell epitopes on the PA toxin component. The experimental approach will be to generate recombinant proteins containing the various domains of PA and to test each individually for their immunogenicity and ability to elicit toxin-neutralizing antibodies in four genetically diverse strains of mice. The original Aims of this award were: 1) to clone, express, and purify the four protein domains of *B. anthracis* protective antigen in amounts suitable for immunization studies; 2) to identify the relative and effective immunogenicity of the four protein domains of *B. anthracis* protective antigen; and 3) to establish the relative protective efficacy of serum elicited by the four protein domains of *B. anthracis* protective antigen. These studies were planne to yield information that could be used to design an effective 3rd-generation vaccine for protection against anthrax.

Body

The project has moved rapidly from what was proposed in the original application. Initially we proposed to essentially break up PA and find out which domains were most immunogenic and effective at eliciting protective antibodies. A good amount of that work was completed as we were waiting for this application to be funded as is described in the publication listed below. Perhaps the greatest surprise of the domain immunization study was the observation that Domain 1 was highly immunogenic and elicited protective antibodies. This was in contradistinction to what was thought in the literature which tended to emphasize Domain 4 as the key domain for targeting the immune response.

In an effort to generate a fine specificity antigenic map of PA we constructed a peptide ELISA containing overlapping peptides to encompass the entire region of

PA. Using this ELISA we identified several domains that are reactive with immune sera. Considerable work went into the development of this ELISA which relies on the synthesis of biotinylated peptides and their attachment to avidin coated plates. By careful attention to various conditions we were able to design a sensitive, low background ELISA suitable for the identification of antibodies to liner PA epitopes. We then used this ELISA to map the fine specificity of several monoclonal antibodies generated in our laboratory. To our pleasant surprise, several of these mAbs reacted with apparently linear epitopes that encompassed the furin cleavage site in Domain 1. The identification of linear epitopes that can elicit protective antibodies is a very exciting new development because it could greatly simplify the approach to a new type of vaccine against PA. Basically, if you can isolate the sequences that elicit useful antibodies and make them into a vaccine one can avoid the problems with whole PA molecule immunization including its capacity to elicit disease-enhancing antibodies.

In the past few months we have made good progress in generating a novel vaccine for protection against anthrax, which is the overall goal of the research award. Using a peptide ELISA described in the past progress report we mapped the peptides recognized by protective monoclonal antibodies. The peptides were then attached to a carrier and used to immunize mice. We are happy to report that the peptides are immunogenic. Preliminary studies indicate that the serum generated from the peptide immunized mice protects against Lethal toxin. This is very exciting because it suggests that we have isolated a region of the PA molecule that retains immunogenicity and can elicit a protective antibody response, at least in mice. We are currently testing three peptides and plan to see how these differ in immunogenicity and ability to elicit protective antibody responses in different mouse strains (Figure 1).

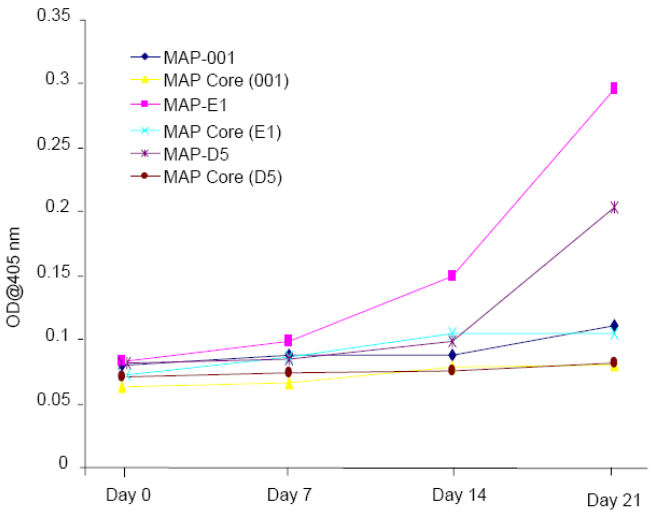

Figure 1: **BALB/c mice were immunized with 100 µg of MAP–LKQKSSNSRKKRSTS (D5), MAP-VKNKRTFLSPWISNI (E1) or MAP-IRLEKGRLYQIKIQY (001) in CFA on day 0, and boosted with MAP–peptide in IFA on days 7 and 14. Control mice were immunized with MAP core in adjuvant. Sera from the different time points were diluted 1:500, and assayed for IgG antipeptide antibodies by ELISA. Data represent averages of five mice receiving MAP–peptide in adjuvant and five mice receiving MAP core in adjuvant**

We are continuing the studies on epitope mapping and are trying to sort out the contribution of isotype to toxin neutralization. The latter is important since our preliminary results indicate that the antibody response to the PA is isotype

restricted with a predominance of IgG1 mAbs. In addition, we have established collaboration with the New York Structural Biology center to solve the solution structure of PA20. This will give us solution structural information that could be extremely useful in identifying the most suitable peptides for vaccine development.

Key Research Accomplishments

- Established the relative immunogenicity of intact PA and between certain domains of PA (published)
- Established the presence of protective epitopes on Domain 1 of PA
- Developed a sensitive ELISA expressing the overlapping domains of PA peptide sequences that have allowed up to identify several linear protective epitopes of PA
- Identified the first linear protective epitopes of PA
- Developed a candidate peptide-based vaccine employing linear epitopes of PA that is immunogenic in mice.

Reportable Outcomes

The data included as preliminary data in the original application was accepted for publication and published.

Abboud N, Casadevall A (2008) Immunogenicity of Bacillus anthracis protective antigen domains and efficacy of elicited antibody responses depend on host genetic background. Clin. Vaccine Immunol. 15:1117-23

Conclusion

The identification of linear peptides in PA that have the potential to elicit protective antibodies could herald a new approach for vaccine development to protect our service men against biological warfare. In contrast to the problems with the current AVA vaccine and recombinant PA, a peptide based vaccine would be chemically defined and that could translate into greater stability, safety and efficacy. In the coming year we plan to test candidate vaccines in mice and by the conclusion of this award hope identify the most suitable sequences for possible clinical vaccine development.

Appendices

None